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Prevalence of TT Virus DNA in Eastern Taiwan Aborigines

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We studied the prevalence of TT virus (TTV) DNA in the general population of the eastern Taiwan aborigine villages, about 11% (34 of 317). There is no association between the presence of HBsAg and TTV DNA or betwen the presence of HCV RNA and TTV DNA. Therefore, the infection of HBV or HCV and the presence of TTV DNA appear to be independent from each other. The association between the presence of TTV DNA and the elevated alanine aminotransferase (and/or aspartate aminotransferase) activity was also investigated. The presence of TTV DNA was not found to be correlated with abnormal liver function ($P = 0.574$) when age, gender, and the presence of HBsAg, HCV RNA, and HGV RNA were all considered in the assay. The sequence homology of TTV DNA fragments between different isolates from Taiwan and N22 (the clone obtained from the original prototype strain) from Japan ranged from 84 to 97%. The recombinant protein encoded by the TTV DNA fragment corresponding to the open reading frame of N22 was expressed in *E. coli* successfully. However, no serum response against the recombinant protein was detected. ***J. Med. Virol. 59: 198–203, 1999.***

KEY WORDS: TT virus (TTV); hepatitis viruses; alanine aminotransferase (ALT); aspartate aminotransferase (AST); open reading frame

INTRODUCTION

Though many types of hepatitis (A–G) are known, there are still patients with acute or chronic hepatitis of unknown etiology [Wright et al., 1991; Fagan, 1994], such as infection of TT virus [Nishizawa et al., 1997].

Recently, an N22 clone (the viral gene fragment of TTV) was isolated from the serum of a patient with posttransfusion non-A to G hepatitis by means of representational difference analysis [Nishizawa et al., 1997]. TTV, with a density of 1.31–1.32 g/cm^3, is a nonenveloped, single-stranded DNA virus, which resembles the *Parvoviridae* [Okamoto et al., 1998a]. The amount of TTV DNA detected in the liver cells is 10 to 100 times higher than that in the corresponding serum from the same patients [Okamoto et al., 1998a], suggesting that TTV may replicate in the liver cells.

In Japan, the TTV DNA is detected more frequently in patients with acute (9/19, 47%) and chronic (41/90, 46%) hepatitis of non-A–G infection than in blood donors (34/290, 12%) [Okamoto et al., 1998a]. The transmission of TTV is likely by blood transfusion or via fecal-oral routes [Okamoto et al., 1998b]. In Thailand, the prevalence of TTV is 38/105 (36%) in healthy blood donors and 55/127 (43%) in the patients with liver diseases, including chronic hepatitis and hepatocellular carcinoma [Tanaka et al., 1998]. Therefore, it has been suggested that TTV may play a role in the development of chronic liver diseases of unknown etiology in Thailand. In Germany, TTV DNA was also detected in 16/84 (19%) patients with liver cirrhosis, and in 4/25 (16%) patients of non-A–G hepatitis [Hohne et al., 1998].

Hepatitis A, B, and C viruses are prevalent in the eastern Taiwan aborigines where the majority of Taiwan aborigines reside (Lee et al., personal communication). However, the prevalence of TTV DNA in the same population is still unknown. This has prompted us to investigate the association between the presence of TTV DNA and the development of hepatitis in the general population of this area. In addition, in order to study the serologic response toward TTV infection, we have expressed the TTV DNA fragments corresponding

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to the open reading frame of N22 in *E. coli* for immunological analysis.

MATERIAL AND METHODS

Study Population and Serum Samples

The data for this investigation were obtained from those of a large-scale community-based cohort study of alcohol-related health problems among aborigines in Hualien, Taiwan (Lee et al., personal communication). Subjects were recruited from two Atayal villages, Fushiu and Sholin, from July 1996 through June 1998. All persons at the age of 15 years and older who resided in the selected communities were invited to participate. The surveillance consisted of two components: a questionnaire and a set of laboratory tests. In the first stage of the study, several trained interviewers carried out a door-to-door survey in the two villages. The contents of the survey included sociodemographic characteristics, disease history, and life style (such as smoking, betel nut chewing, vehicular safety, diet, and alcohol use). On the second stage of the investigation, a team of investigators including a physician and several nurses conducted free health check-up programs and collected samples of blood and urine in the selected communities. During the enrollment period for the cohort study, 2,169 screening questionnaires were carried out. Of these cases, 1,061 (48.9%) consented to have a physical exam and blood sampled. Persons who agreed to have their blood tested tended to be older than those who refused to participate (mean age, 43 vs. 34 years, $P < .001$). There were more female subjects in the enrolled group than those in the nonenrolled (54.0% vs. 46.9%, $P < .001$). All blood samples were collected in a portable refrigerator, delivered to the central laboratory for serum separation, and stored at −70°C until further analysis.

The present investigation focused on a sample of 317 subjects, which were randomly selected from the larger study population (n = 1,061). Of the 317 study cases, 283 (89.3%) were Atayal. One hundred and eighty-six (58.7%) were women, and the average age was 45 years old.

Nucleic Acid Extraction

The nucleic acid was extracted from sera using either the "AcuPure DNA/RNA Extraction Kit" (Biotronics Tech. Corp., Lowell, MA) or the Guanidium thiocyanate method described previously [Lo et al., 1994]. After nucleic acid extraction, the nucleic acid was used directly for PCR for the detection of TTV DNA or for cDNA synthesis by using random hexamer as the primer for the analysis of HCV or HGV RNA.

Polymerase Chain Reaction (PCR)

The primers and condition used in the nested PCR for the detection of HGV RNA were the same as those used to analyze the 5′UTR of HGV [Kao et al., 1997]. The detection of HBsAg, anti-HBs, and anti-HCV will be described elsewhere (Lee et al., personal communication). The "uPlate Anti-HGVenv" kit from Boehringer Mannheim was used for the anti-HGV assay. The detection of HCV RNA is using the primers located in the 5′-UTR to perform the nested PCR.

The nested PCR primers and the PCR program for the detection of the 197 bp TTV DNA fragment were the same as those used before [Nishizawa et al., 1997]. If the samples were positive in the nested PCR, a semi-nested PCR was carried out to amplify a 396 bp TTV DNA for sequencing. The semi-nested PCR primers and the PCR program were the same as described [Nishizawa et al., 1997].

The primers for cloning the gene fragment for expression in *E. coli* were the sense primer, 5′GGAATTC<u>CATATG</u>ACACCAGGAGCATAT3′ and the anti-sense primer, 5′CG<u>GGATCC</u>**CTA**TTCTTGCTGGTGAAA3′ (the underlined nucleotides are the restriction sites while the bolded nucleotides are the stop codon). After amplification, the PCR products were cloned into the Nde I and Bam HI sites of the pET3a expression vector. The expression of the recombinant protein is initiated from the ATG in the designed Nde I site and terminated by the stop codon (TAG) right before the Bam HI site.

Sequencing and Analysis

The PCR products were cloned into the vector pBlueScript-SK(+). The plasmids were extracted by using the "Wizard Plus Minipreps" kit (Promega, Madison, WI). The "ABI PRISM™ Terminator Cycle Sequencing Ready kit" (Perkin-Elmer, Oak Brook, IL) was used to perform the automatic sequencing assay. The procedure and condition were the same as described in the manufacturer's instructions. Both M13 reverse primers and T7 primers were used to sequence the plasmids. At least three clones were sequenced to determine the consensus sequence for each PCR product.

The nucleotide sequences were analyzed by using the GCG system provided by the National Health Research Institute, Taiwan (Republic of China).

Protein Expression and Western Blot

The procedure used in protein expression has been described previously [Lo and Ou, 1998]. Briefly, *E. coli* BL21(DE3)pLysS cells transformed by pET3a control plasmid or expression plasmids containing the inserts (TTV41 and TTV162) were grown to the stationary phase. A 1-ml culture of the stationary phase cells was inoculated into a fresh 100 ml culture and grown to O.D. 0.6. IPTG was then added to the culture to a final concentration of 1 mM. After further incubation at 37°C for 3 hours, *E. coli* cells were pelleted by centrifugation. The pellets from 300 μl culture was dissolved in Laemmli buffer, boiled, and loaded on two 12.5% SDS-PAGE gels. One gel was for staining and the other was for Western blotting. The procedure for Western blotting was as described previously [Lo et al., 1995]. After electrophoresis, the gel was Western-blotted to nitrocellulose (NC) paper. All steps were done at room tem-

perature. The NC paper was blocked in TBS (10 mM Tris, pH 7, and 150 mM NaCl) with 5% milk for 3 hours. After blocking, the NC paper was incubated with the primary antibody, which had been diluted 1,000-fold in TBS containing 0.1% NP-40 and 1% milk, for 3 hours. The primary antibodies used were sera isolated from persons in this study. The NC paper was then washed three times in TBS for 10 minutes. Afterwards, the goat anti-human IgG conjugated with alkaline phosphatase (Sigma Chemical Co., St. Louis, MO), which had been diluted 1,000-fold in TBS containing 0.1% NP-40 and 1% milk, was added for another 1 hour of incubation. After three more 10 minute washes with TBS, the signal was developed by the AP conjugate substrate kit (Bio-Rad, Richmond, CA).

Statistical Analysis

Descriptive statistics such as means and proportions were calculated. Subgroup responses were compared by the χ^2 test for categorical variables and Student t test, for continuous variables. A two-tailed *P* value less than .05 was considered statistically significant for all pairwise comparisons. Multiple logistic regression analysis [Hosmer and Lemeshow, 1989] was used to assess the independent association between the presence of TTV DNA and an abnormal liver function, while controlling for other possible factors, such as gender, age, and the presence of HBsAg, HCV RNA, and HGV RNA. Odds ratios (ORs) and their associated 95% confidence intervals (CIs) were used to quantify the magnitude of these associations. Statistical analyses were performed using SAS statistical software [SAS Institute, Inc., 1997].

RESULTS

Prevalence of TTV DNA

TTV DNA (197 bp, detected by nested PCR, data not shown) were present in 34 (10.7%) of the 317 studied samples. TTV DNA was found in 11 (8.4%) of the male participants and in 23 (12.4%) of the female subjects. There is no significant difference in the distributions of TTV DNA between the two gender groups ($\chi^2 = 1.26$, 1 df, $P = 0.26$). In addition, stratified subjects by three age groups, 15–29, 30–59, and ≥60 years old, reveal no significant differences in the distributions of TTV DNA among the three age groups ($\chi^2 = 0.16$, 2 dfs, $P = 0.92$). TTV DNA was found in 9 (11.8%), 17 (10.6%), and 8 (9.9%) of the three age groups, respectively.

HBsAg, HGV RNA, and HCV RNA were present in 72 (22.7%), 17 (5.4%), and 80 (25.2%) of the studied samples, respectively. Proportions of the subjects with the presence of HBsAg and HCV RNA in the TTV DNA (+) and TTV DNA (−) groups are depicted in Table I. The χ^2 analysis indicated there is no association between the presence of HBsAg and TTV DNA or between the presence of HCV RNA and TTV DNA.

Of the 317 samples analyzed, 130 (41.0%) most likely had abnormal liver function, as they had elevated level of serum alanine amino-transaminase (ALT > 30 IU/L) and/or serum aspartate amino-transaminase (AST > 30IU/L). Table II shows the results of multiple logistic regression analysis of abnormal liver function, and adjusted odds ratios and their corresponded 95% confidence intervals for each predictor. The analysis identified two risk factors that were independently associated with abnormal liver function: male gender (OR, 2.2; 95% CI, 1.4–3.5) and the presence of HCV RNA (OR, 1.7; 1.0–2.9). Nevertheless, the presence of TTV DNA was not correlated with abnormal liver function ($P = 0.574$) when age, gender, and the presence of HBsAg, HCV RNA, and HGV RNA were all controlled in the model.

TABLE I. Associations Between the Presence of HBsAg, HCV RNA, and TTV DNA*

	TTV (+) (n = 34)		TTV (−) (n = 283)	
	Number	%	Number	%
HBsAg(+)	8	23.5	64	22.6
	$\chi^2 = 0.01$, 1 df, $P = 0.90$			
HCV RNA (+)	9	26.5	71	25.1
	$\chi^2 = 0.03$, 1 df, $P = 0.86$			

*TTV, TT virus; HCV, hepatitis C virus; HBsAg, hepatitis B virus surface antigen.

TABLE II. Predictors of Abnormal Liver Function: Multiple Logistic Regression Analysis*

	% of cases with abnormal liver function	Adjusted OR*	95% CI*	P-value
TTV				
Positive	35.3	0.8	0.4–1.7	0.574
Negative	41.7	1.0		
Age group				
15–29	32.9	1.1	0.5–2.2	0.827
30–59	46.9	1.6	0.9–2.8	0.128
≥60	37.0	1.0		
Gender				
Male	52.7	2.2	1.4–3.5	0.001
Female	32.8	1.0		
HBsAg				
Positive	44.4	1.2	0.7–2.2	0.467
Negative	40.0	1.0		
HCV RNA				
Positive	48.8	1.7	1.0–2.9	0.062
Negative	38.4	1.0		
HGV RNA				
Positive	35.3	0.9	0.3–2.7	0.910
Negative	41.3	1.0		

*OR, Odds Ratio; CI, Confidence Interval.

Sequence Diversity

All 34 TTV DNA positive samples that tested positive by nested PCR (197 bp, data not shown) also tested positive by semi-nested PCR (396 bp, data not shown). Six randomly selected DNA samples were sequenced. The sequence of each serum sample was determined from the consensus sequence of at least three clones. The sequence homology of TTV DNA fragments between different isolates from Taiwan and N22 from Ja-

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N22   GGAGCATATACAGACATAAAGTACAATCCATTCACAGACAGAGGA
041   ---------------------------------------------
177   --------------------A-----------T------------
222   --------------------A-----------T------------
162   -----------------C--A--T--C--T--T------------
161   -----------------C--A--T--C--T--T------------
140   -----------------C--A--T--C--T--T------------

N22   GAAGGCAACATGTTATGGATAGACTGGCTAAGCAAAAAAAACATG
041   ------------C--------------------------------
177   ------------T---------------------------C----
222   ------------T-------------------------G-A----
162   ------------C-G--------------T-CT-----A-A----
161   ------------C-G--------------T-CT-----A-A----
140   ------G-----C-G--------------T-GT-----A-A----

N22   AACTATGACAAACTACAAAGTAAATGCTTAATATCAGACCTACCT
041   ------------G--------------------------------
177   ------------G-----------------G--------------
222   ------------G-----------------G--------------
162   G-A---------G-G-----C--G---C--G--G----------A
161   G-A---------G-G-----C--G---C--G--G----------A
140   G-A---------G-G-----C--G---C--G--G------C---A

N22   CTATGGGCAGCAGCATATGGATATGTAGAATTTTGTGCAAAAAGT
041   ---------------------------------------------
177   ---------------------------------------------
222   ------------------------T-------C--CT-T-----C
162   --G---------------------T-------C--CT-T-----C
161   --G---------------------T-------C--CT-T-----C
140   --G-----------------G---T-------C--CT-T-----C

N22   ACAGGAGACCAAAACATACACATGAATGCCAGGCTACTAATAAGA
041   ---------------------------------------------
177   -----------G---------------------------------
222   ---------ACA--------------------A--G---------
162   ---------ACA--------------------A--G---------
161   ---------ACA--------------------A--G---------

140   ---------ACA--------------------A--G---------

N22   AGTCCCTTTACAGACCCACAACTACTAGTACACACAGACCCCACA
041   ---------------------------------------------
177   ---------------------------------------------
222   -----------------C--G---A-----------A------AT
162   -----------------C--G---A-----------A------AT
161   -----------------C--G---A-----------A------AT
140   -----------------C--G---A-----------A------AT

N22   AAAGGCTTTGTTCCTTACTCTGTAAACTTTGGAAATGGTAAAATG
041   ---------------------T-----------------------
177   ---------------------T-----------------------
222   -----------A-----T---T-----------------------
162   -----------A-----T---T-----------------------
161   -----------A-----T---T-----------------------
140   -----------A-----T---T----T--------------G---

N22   CCAGGAGGTAGTAGTAATGTGCCTATTAGAATGAGAGCTAAATGG
041   ---------------------------------------------
177   ---------------------------------------------
222   --------------C--C--T--C--A-----------A--G---
162   --------------C--C--T--C--A-----------A--G---
161   --------------C--C--T--C--A-----------A--G---
140   --------------C--C--T--C--A-----------A--G---

N22   TATCCAACATTATTTCACCAGCAA
041   ------------------------
177   ------------------------
222   --C--C--TC--------------
162   --C--C--TC--------------
161   --C--C--TC--------------
140   --C--C--TC--------------
```

Fig. 1. Sequence comparison of TTV DNAs between different Taiwanese isolates and the N22 clone [Nishizawa et al., 1997] from Japan. Dash-lines represent nucleotides identical to those in the N22 clone.

pan ranged from 84 to 97% (Fig. 1). The deduced amino acid homology (Fig. 2) between different isolates from Taiwan and N22 from Japan ranged from 88 to 98%.

The N22 Gene Fragment May Not Encode Viral Proteins

We have expressed the corresponding gene fragments of two different isolates (TTV41 and TTV162) in *E. coli* for serologic studies. Both DNA fragments could encode a polypeptide approximately 15 kDa in size (Fig. 3). The serologic responses to these polypeptides were analyzed by Western blotting. Five sera positive for TTV DNA and another twenty-eight TTV DNA negative sera were randomly selected and used as the primary antibodies in the assay. None of them reacted positively with the expressed protein in the assay (data not shown). Furthermore, ten more specimens were selected to perform the Western blot. These ten specimens were derived from paired sera of five convalescent individuals. One specimen of the paired sera was collected when TTV DNA was detected in the persons, while the other one was obtained later when the TTV DNA was undetectable. However, none of these ten specimens showed positive signal in the assay (data not shown).

```
N22   IKYNPFTDRGEGNMLWIDWLSKKNMNYDKLQSKCLISDLP
041   -----------------------------V----------
177   -----------------------------V-----V----
222   -----------------------------V-----V----
162   --------------------T----E---V-----VA---
161   --------------------T----E---V-----VA---
140   ------------D-------S----E---V-----VA-P-

N22   LWAAAYGYVEFCAKSTGDQNIHMNARLLIRSPFTDPQLLV
041   ----------------------------------------
177   ----------------------------------------
222   --------L---S-----T-------------------I-
162   --------L---S-----T---------T---------I-
161   --------L---S-----T-------------------I-
140   --------L---S-----T-------------------I-

N22   HTDPTKGFVPYSVNFGNGKMPGGSSNVPIRMRAKWYPTLF
041   ------------L---------------------------
177   ------------L---------------------------
222   --N-N-------L---------------------------
162   --N-N-------L---------------------------
161   --N-N-------L---------------------------
140   --N-N-------L---------------------------
```

Fig. 2. Comparison of deduced amino acid sequence of TTVs between different Taiwanese isolates and the N22 clone [Nishizawa et al., 1997] from Japan. Dashed-lines represent amino acids identical to those in the N22 clone.

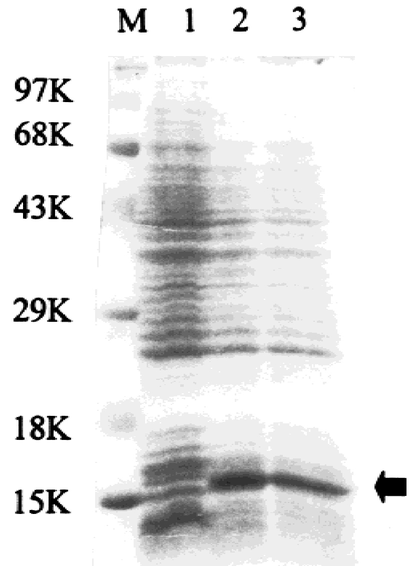

Fig. 3. Expression of TTV DNA fragments from two different isolates in *E. coli*. Details of the expression procedures are described in Materials and Methods. After induction, the *E. coli* BL21(DE3)pLysS cells transformed by pET-3a (lane 1), pET-TTV41 (lane 2), or pET-TTV162 (lane 3) were pelleted by centrifugation. The pellet was dissolved in Laemmli buffer, boiled and loaded on the SDS-PAGE gel. The gel was then stained with Coomassie blue.

DISCUSSION

We reported here the prevalence of TTV DNA in the general population of eastern Taiwan aborigines, which is about 11% (34 of 317), similar to that of the blood donors (12%) in Japan [Okamoto et al., 1998a]. The association between the presence of TTV DNA and the elevated ALT (and/or AST) value was also studied (Table II). The presence of TTV DNA was not correlated with abnormal liver function ($P = 0.574$) when age, gender, and the presence of HBsAg, HCV RNA, and HGV RNA were all controlled in the model. These data suggest that the presence of TTV DNA may not cause the elevation of ALT (and/or AST) value in the eastern Taiwan aborigines.

The association between HCV (or HBV) infection and the presence of TTV DNA was also analyzed (Table I). The frequency of the presence of TTV DNA alone (20/34, 59%) is similar to that (23/39, 59%) in Thailand [Tanaka et al., 1998]. There is no associatioin between the presence of HBsAg and TTV DNA or between the presence of HCV RNA and TTV DNA. Therefore, the infection of HBV (or HCV) and the presence of TTV DNA appear to be independent from each other. The amount of TTV DNA detected in the liver cells is 10 to 100 times higher than that in the serum of the same patients [Okamoto et al., 1998a]. Co-infection of TTV with HBV and/or HCV is also frequently detected (14/34, 41%) in eastern Taiwan aborigines. Whether TTV may affect the replication of HBV or HCV in liver cells requires further investigation.

At present, 3,739 bases (accession number AB008394) of TTV DNA sequences have been determined which contain two possible open reading frames encoding 770 (ORF 1) and 202 (ORF 2) amino acids. The DNA fragment of N22 clone belongs to part of ORF1 [Okamoto et al., 1998a]. Only one out of six possible reading frames from either strand of N22 DNA fragment can encode polypeptide of more than 100 amino acids [Nishizawa et al., 1997]. In order to study the serologic response toward TTV infection, we have successfully expressed the open reading frame corresponding to the N22 clone in *E. coli* (Fig. 3). Five random TTV DNA-positive sera and twenty-eight sera negative for it were used for Western blot analysis to detect the presence of antibodies against this recombinant protein. None of them had positive signal (data not shown). It is possible that the antibody against this ORF does not coexist with TTV DNA like anti-E2 and HGV RNA in HGV infection. Therefore, ten more specimens were selected to perform the Western blot. These ten specimens were derived from paired sera of five convalescent individuals. One specimen of the paired sera was collected when TTV was detected in the persons while the other one was obtained later when the TTV DNA was undetectable. However, none of these ten specimens reacted positively in this assay (data not shown). These data suggest that the ORF of N22 clone (part of TTV ORF1) may not encode any viral proteins or this ORF product in the patients is not very immunogenic.

To understand more about the serologic response and etiology of TTV, the full-length genomic sequence of this virus should be determined.

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REFERENCES

Fagan EA. 1994. Acute liver failure of unknown pathogenesis: the hidden agenda. Hepatology 19:1307–1312.

Hohne M, Berg T, Muller AR, Schreier E. 1998. Detection of sequences of TT virus, a novel DNA virus, in German patients. J Gen Virol 79:2761–2764.

Hosmer DW and Lemeshow S. 1989. Applied logistic regression. New York: Wiley.

Kao J, Chen P, Chen W, Hsiang S, Lai M, Chen D. 1997. Amplification of GB virus/Hepatitis G virus RNA with primers from different regions of the viral genome. J Med Virol 51:284–289.

Lo S, Ou J. 1998. Expression and dimerization of hepatitis C virus core protein in *E. coli.* In: Lau JYN. Methods in molecular medicine, Vol 19: Hepatitis C protocols. Totowa, NJ: Humana Press, Inc.; p 325–330.

Lo S, Selby M, Tong M, Ou J. 1994. Comparative studies of the core gene products of two different hepatitis C virus isolates: two alternative forms determined by a single amino acid substitution. Virology 199:124–131.

Lo S, Masiarz F, Hwang SB, Lai MM, Ou J. 1995. Differential subcellular localization of hepatitis C virus core gene products. Virology 213:455–461.

Nishizawa T, Okamoto H, Konishi K, Yoshizawa H, Miyakawa Y, Mayumi M. 1997. A novel DNA virus (TTV) associated with elevated transaminase levels in posttransfusion hepatitis of unknown etiology. Biochem Biophys Res Commun 241:92–97.

Okamoto H, Nishizawa T, Kato N, Ukita M, Ikeda H, Iizuki H, Miyakawa Y, Mayumi M. 1998a. Molecular cloning and characterization of a novel DNA virus (TTV) associated with posttransfusion hepatitis of unknown etiology. Hepatol Res 10:1–16.

Okamoto H, Akahane Y, Ukita M, Fukuda M, Tsuda F, Miyakawa Y, Mayumi M. 1998b. Fecal excretion of a nonenveloped DNA virus (TTV) associated with posttransfusion non-A-G hepatitis. J Med Virol 56:128–132.

SAS Institute Inc. 1997. SAS/STAT Software: changes and enhancements through release 6.12. Cary, NC: SAS Institute Inc.

Tanaka H, Okamoto H, Luengrojanakul P, Chainuvati T, Tsuda F, Tanaka T, Miyakawa Y, Mayumi M. 1998. Infection with an unenveloped DNA virus (TTV) associated with posttransfusion non-A to G hepatitis in hepatitis patients with healthy blood donors in Thailand. J Med Virol 56:234–238.

Wright TL, Hsu H, Donegan E, Feinstone S, Greenberg H, Read A, Ascher NL, Roberts JP, Lake JR. 1991. Hepatitis C virus not found in fulminant non-A, non-B hepatitis. Ann Int Med 115:111–112.